Is it possible to increase bioavailability but not environmental risk of PAHs in bioremediation?

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ABSTRACT

The current poor predictability of end points associated with the bioremediation of polycyclic aromatic hydrocarbons (PAHs) is a large limitation when evaluating its viability for treating contaminated soils and sediments. However, we have seen a wide range of innovations in recent years, such as an the improved use of surfactants, the chemotactic mobilization of bacterial inoculants, the selective biostimulation at pollutant interfaces, rhizoremediation and electrobioremediation, which increase the bioavailability of PAHs but do not necessarily increase the risk to the environment. The integration of these strategies into practical remediation protocols would be beneficial to the bioremediation industry, as well as improve the quality of the environment.

Contents

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants present in the vast majority of contaminated areas. They are recognized worldwide as priority pollutants in soils, sediments and waters because of their toxicity and carcinogenicity. Industrial and urban emissions are contributing sources of PAHs to the atmosphere, which can be transferred at least partially to soils and sediments. Elevated PAH concentrations have been detected in soils found near industrial activities [1, 2], road traffic [3] and accidental fires [4], resulting in the increase in PAH concentrations found in both industrial and agricultural soils over the last century [5, 6]. The anthropogenic emissions of PAHs can also be transported over long distances and introduced into soils and sediments through dry or wet deposition at more remote places, such as high-altitude lake areas [7]. The background concentrations of PAHs in soils differ according to the geographical characteristics and size of the area under study, but they often range between 100 and 1,000 μg/kg. Higher PAH concentrations in soils are usually found in soils that exhibit point-source pollution derived from accidental spills or the disposal of industrial wastes [8-10]. Unacceptably high PAH concentrations in soils are the main reason for the remediation of many contaminated sites [11].

PAHs are among the most commonly found organic contaminants that have significant potential for bioremediation. However, PAHs are the best representatives of chemicals for which specific limitations in bioremediation exist due to low bioavailability. The residual concentrations of the PAHs after bioremediation are crucial because they may limit the use of the area after treatment, or land use might not even be possible if the residual concentrations do not meet the legal requirements. Therefore, innovative methods are required to increase the bioavailability of these pollutants to

achieve an enhanced bioremediation performance. For these innovations to be competitive, they should lead to increased biodegradation rates and lower residual concentrations, but they should also keep associated risks at reasonably low levels. The concepts presented in this review constitute a summary of the most recent efforts carried out in the bioremediation field to understand the interconnections of the bioavailability and biodegradation of PAHs.

2. Biodegradation and bioremediation of PAHs

Biodegradation reactions carried out by many bacteria and fungi constitute one of the most direct pathways for the dissipation of PAHs in natural environments [12]. Biodegradation is dependent on the stability of the benzene rings in the PAH molecules and on other factors related to their hydrophobicity, which is the source of their strong tendency to sorb onto solid surfaces, such as clays and organic matter [12]. Another characteristic of these chemicals is that they can typically be found in complex materials. These materials include nonaqueous-phase liquids (NAPLs), such as creosote and coal tar, and soot-like materials, which are generally known as black carbon (BC). The biodegradation of PAHs found in BC or NAPLs is typically a slow process that contributes to their environmental recalcitrance [13-15]. These chemicals consequently tend to remain in the environment for long periods of time and are considered typical examples of persistent organic pollutants. The half-life of low-molecular-weight (LMW) PAHs in the environment, such as phenanthrene, ranges from 16-126 days, but high-molecular-weight (HMW) PAHs, such as benzo(a)pyrene, possess substantially longer half-lives of up to 1400 days [16]. These half-life estimations take into account the reduction in PAH concentrations not only from biodegradation, but also from other

processes, such as volatilization, photoxidation and chemical oxidation, which remove LMW PAHs more rapidly than HMW PAHs.

The biochemical pathways for attacking PAH structures by microorganisms was established in the 1980s for LMW PAHs, and later (in the 1990s) for HMW-PAHs. The aerobic pathways of PAH metabolism are perhaps the best-known. Three different enzymatic mechanisms are classically accepted [16-19]: i) aromatic ring oxidation with dioxygenases, exclusive to prokaryotic organisms and usually part of the process for the utilization of the substrate as a carbon and energy source, although it may also lead to cometabolic processes; ii) oxidation by means of lignin and manganese peroxidases excreted by wood white-rot fungi, a process of cometabolic nature, although it may result on substrate mineralization; and iii) oxidation with cytochrome P450 monooxygenase, present in both prokaryotic and eukaryotic organisms, and commonly associated to detoxification and later excretion of the metabolites formed, and, therefore, it does not usually entail the mineralization of the initial substrate. The current knowledge on the biodegradation of PAHs indicates that of the 16 PAHs listed by the US-EPA as priority pollutants, those with a molecular weight of up to 202 g/mol can be degraded through growth-linked aerobic reactions, including the HMW-PAHs pyrene and fluoranthene, while the rest of the PAHs, such as benzo[a]pyrene, are only susceptible to co-metabolic removal [18]. This distinction is relevant because biodegradation reactions based on the aerobic assimilation of PAH molecules into microbial cells are typically faster than those reactions that are based on co-metabolism. The transformations of PAH molecules via co-metabolism do not provide any benefit to the microorganisms that co-metabolize these substrates. These reactions do not cause extensive modifications of the PAH molecules, the incorporation of the substrate carbon into biomass, or the direct conversion of the substrate into CO_2. As a result, the

microorganisms responsible for co-metabolism do not proliferate, resulting in a low rate of biodegradation. Microorganisms co-metabolize these PAHs at the expense of the assimilation of other compounds, including other PAHs, which can be easily identified by experimental models developed in the laboratory but are not so evident in field studies. This can be directly translated into the biodegradability often observed in aerobic environments, such as soils, which shows a high recalcitrance of PAHs composed of five or more benzene rings [20].

The oxygen dependence often observed in the biodegradation of PAHs gave rise to the belief that the anaerobic conditions usually found in certain environments, such as clay-rich soils, waterlogged soils, estuaries, ports, and other coastal zones close to contamination sources, were solely responsible for causing a long persistence of PAH pollutants in those environments. However, recent findings have demonstrated that microorganisms can also use other electron acceptors, such as nitrates and sulfates, to oxidize PAHs in the environment [21, 22]. It was also recently shown that the anaerobic biodegradation of PAHs in certain environments could proceed only when a second carbon source, such as acetate or glucose, was present in the biodegradation environment [23]. According to these studies, the proposed recalcitrance of PAHs in anaerobic environments has to be revisited. In addition, the biodegradation of PAHs at low oxygen tensions may be important at certain environmental boundaries, such as at the interface between the anoxic sediments and the overlying waters [15].

Organic carbon (OC), in the form of solid-phase or “dissolved” organic matter (SOM and DOM, respectively), also plays a key role in the environmental fate of PAHs. Studies on the effect of SOM on biodegradation of PAHs in soil have often evidenced an inhibitory effect. A strong sorption of PAHs to SOM was found to be correlated with a decreased biodegradation of these chemicals in soil [24]. In that study, the authors

found that in a contaminated soil from a coking plant, containing a high percentage of SOM, biodegradation did not occur even after addition of nutrients and PAH-degrading bacteria. However, PAH extraction and addition to the extracted soil material led to a rapid biodegradation. The authors concluded that physical binding or sorption of the PAHs to the soil was the cause for the initial lack of mineralization. A comparison of mineralization rates of phenanthrene in four soils [25] found a clear reduction in the soil with the highest SOM content. It was suggested that this reduction was caused by sorption to SOM. Similar observations about this inhibiting effect of SOM on phenanthrene mineralization in soils were reported later [26]. These authors also showed that the humic acid fraction of a soil SOM was more effective in inhibiting biodegradation than the fulvic acid of the same soil, and explained this effect on the basis of weaker interactions of phenanthrene with the less-hydrophobic fulvic acid fraction. Negative effects on biodegradation in soil due to sorption to SOM have also been shown for naphthalene [27].

Although there are reports on the inhibition of biodegradation of organic chemicals due to binding to DOM [28, 29], there is an increasing body of evidence pointing to an enhancing effect in the case of PAHs. The addition of DOM in the form of humic fractions to PAH-contaminated soils caused an enhancement of biodegradation, probably as a results of the enhanced desorption of these compounds from soils [30-32]. Other mechanisms proposed as operating in DOM-mediated enhancements of biodegradation include the promotion of PAH solubility [33], a direct access to DOM-sorbed PAHs due to the physical association of bacteria and DOM [34], and an increased diffusive flux toward bacterial cells caused by DOM [35]. The latter mechanism would be analogous to that described for the enhanced uptake of metals by

plants in the presence of labile metal complexes, caused by an enhanced diffusional flux through unstirred boundary layers around the roots [36].

During recent years, the one-phase organic-matter partitioning model traditionally used to describe sorption of hydrophobic PAHs in the environment has been expanded to include the high sorption capacity of the ubiquitous, solid-phase product of incomplete combustion known as black carbon (BC). Therefore, both adsorption to BC and absorption by OC would occur in parallel during the sorption process [37-39]. The new model has been useful in understanding field observations of PAHs´ solid-water distribution coefficient (K_d) in sediments, which have evidenced a higher sorption capacity than would have been expected on the basis of OC content only [40, 41]. Various studies have shown that strong sorption of PAHs to BC may also significantly limit biodegradation. For example, 16 USEPA PAHs associated with carbonaceous coal-derived material present in harbor sediments exhibited negligible biodegradation rates in aerobic sediment slurries, whereas similar conditions led to significant losses (up to 75 % after two months) of PAHs present in semisolid coal tar pitch [42]. Little or no biodegradation was also observed for 3- to 6-ringed PAHs associated to BC-rich street dust added to soils to simulate diffuse pollution [43]; for phenanthrene in BC-rich crusts present on building stones from two European cathedrals, as compared with crust-free adjacent zones [44]; and with naphthalene sorbed to granular activated carbon, a material similar to BC in its physicochemical characteristics, in suspensions of two different bacterial species with dissimilar modes of acquisition of the sorbed compound [45]. Finally, Rhodes et al. (2008) examined the effect of BC on bioavailability of phenanthrene in soils. They found that the addition of BC to soils caused a significant decrease both in the final extent of mineralization and in the extractability by cyclodextrin solutions [46].

Due to their charged nature and high specific surface, natural clay minerals also play an important role in the biodegradation of PAHs. Microbial cells usually show a high affinity for clay surfaces, as evidenced by their spontaneous association in suspensions and percolation columns [47-49]. This association can explain, for example, the concentration of a PAH-degrading *Mycobacterium* community in the PAH-enriched clay fraction of a long-term contaminated soil [50]. Clay surfaces can also scavenge organic chemoeffectors from the pore water by sorption, thereby eliminating their effect in promoting the transport of chemotactic bacteria through porous materials [49], and associate with SOM, resulting in slowly desorbing fractions of PAHs with limited bioavailability to microorganisms [51]. Clay-rich soils may also present a limited oxygen and nutrient supply to PAH-degrading populations, due to slow diffusion and low hydraulic conductivity [52].

With our deep knowledge of how PAHs undergo biodegradation in the environment, it is not surprising that the application of bioremediation to the treatment of PAH pollution is also well documented. Today, the price of bioremediation is comparable to that of other options in the remediation market, such as chemical extraction, incineration and thermal desorption [53-56]. Furthermore, bioremediation is advantageous over other technologies with regard to the fact that the soil or sediment can retain many of its key functions in contrast to ex-situ treatment methods and landfill capping/sealing, which may allow it to be used again after treatment, thus providing an economic value to that land as well. For example, the potential for biologically treating sites polluted by creosote, a PAH-rich wood preservative, was recognized in the United States as early as 1989 [57] and was later documented in other parts of the world [58-60]. The variety of approaches that have been used and validated to biologically treat soils and sediments contaminated with PAHs are summarized in Table 1. A

comprehensive review of the biological and non-biological remediation methods suitable for soils polluted with PAHs can be found elsewhere [65].

However, the benefits derived from the biodegradation of these chemicals are also accompanied by the uncertainties that surround the use of bioremediation when the bioavailability of these chemicals remains unpredictable. The current poor predictability of end points associated with the bioremediation of PAHs is a large limitation when evaluating its viability for treating contaminated soils and sediments. To this end, the integration of a bioavailability-efficient technology into current bioremediation practices at no additional cost will ensure that the target values for risk reduction and the cost-effectiveness of the treatment can be achieved. For instance, the disposal of contaminated soil depends on the type of pollution, but it usually costs more than 200 €/m^3. The cost of treating a polluted soil with bioremediation ranges from 20 to 70 €/m^3 (average, 45 €/m^3) [53-56]. Within the European Union, a total of 2.250 billion € has been budgeted for the rehabilitation of industrial sites between 2005 and 2013 [66]. Assuming that 1% of these costs are allocated to PAH-polluted sites comprising 13.3 % of the total number of investigated sites in Europe [55], where bioremediation at a cost of 45 €/m^3 is a realistic alternative to excavation and disposal priced at 200 €/m^3, the potential amount saved by the complete bioremediation of PAHs if bioremediation is preferable to current treatment methods can be approximately 60 billion euros.

3. Contrasting the basic concepts of bioavailability

Bioavailability represents the accessibility of a chemical for biotransformation and toxicity. This concept is difficult to simplify and has recently been the subject of intensive discussion and redefinition [67-69], but it is essential in understanding the significance of biodegradation as one of the main drivers of PAH removal in soils,

sediments and waters. As a result of the partitioning of PAHs into sorbents and NAPLs, they may only exhibit weak chemical activity gradients that promote their uptake and transformation by active microbial cells. Thus, the biodegradation rates may reflect the dependencies of restricted phase exchanges, and the originally present PAHs in the system may persist for longer periods of time. Bioremediation may be effective in reducing the environmental risk incurred by PAHs [70-72], but recalcitrant pollutants left as residuals from bioremediation may still be toxic to specific ecological targets, such as terrestrial invertebrates [73]. Furthermore, under some circumstances bioremediation may even increase bioavailability and risk of PAHs. This is suggested by a study where two different bioassays were employed, in combination with chemical analysis, to evaluate the toxicity in a soil undergoing large-scale bioremediation [74]. The results indicate that, as a result of treatment during 274 days, the soil might have increased its toxic potential to organisms, possibly due to the release of sorbed PAHs and the formation of toxic metabolites. The authors suggested that a prolonged treatment would help to minimize risk of remaining mobile PAHs. Recently, it was reported that treatment of field-contaminated soil from a former manufactured-gas plant site yielded significant reductions in residual PAH levels, but different toxicity and genotoxicity potentials, depending on the method of treatment used [75]. Treating the soil in a slurry-phase bioreactor resulted in an increase in toxicity and genotoxicity over the course of a treatment cycle (7 days), whereas long-term column treatment during 2.5 years resulted in a decrease in toxicity and genotoxicity. The authors did not address yet bioavailability issues in this specific study, but it is possible that the higher bioavailability regime caused by the bioreactor operation contributed to the higher toxicity.

A useful indicator of bioavailability is the fraction of potentially biodegradable PAHs over time in the absence of limitations other than restricted phase exchanges. This is also known as the bioaccessible fraction [68]. The incorporation of a time dimension into bioaccessibility allows it to describe the kinetic nature of the conflict between bioavailability and biodegradation. Another bioavailability indicator is the chemical activity of PAHs, which is the fraction of the aqueous solubility of the PAHs in subcooled liquid state that can be measured as freely dissolved chemical concentrations (C_{free}) in the aqueous phase of a environmental sample [69]. The chemical activity of the PAHs determines their distribution throughout different phases. For instance, the chemical activity of a contaminant in a soil or sediment is a driving force for the diffusion of the contaminant into microorganisms and may therefore determine the biodegradation rate of that contaminant. Chemical activity may also affect the equilibrium partitioning concentration of the contaminant in lipid membranes, which is the reference point for baseline toxicity. It is possible that most biodegradation studies that have been reported to date have used different forms of bioaccessibility estimations rather than chemical activity to characterize bioavailability restrictions due to methodological reasons. However, both aspects are certainly complementary and necessary for a complete understanding of the interconnections between bioavailability, biodegradation and the environmental risk of PAHs.

The biodegradation of PAHs that have been present in the environment for a long time can be a slow process. Aged PAHs can still be extracted by vigorous solvent extraction, but they possess a lower bioavailability and risks compared with the more recently introduced pollutants [67]. Chemicals may also be bound in a residue that does not allow any form of extraction to occur. Bound residues include compounds in soils, plants, or animals that persist in the matrix in the form of the parent substance or its

metabolite(s) after extraction [76, 77]. The extraction method must not substantially alter the compounds themselves or the matrix structure. Therefore, bound residues include chemically unchanged pollutants that are trapped by soil materials, and pollutants which have undergone chemical reactions and have become covalently bonded to or undergone complexation reactions with materials present in the soil. The formation of bound residues may decrease the risk compared with that imposed by the unbound chemicals, but these residues also lead to a higher biodegradation resistance and recalcitrance. Bound PAH residues can be formed not only as a result of their direct chemical association with soil and sediment components but also as a result of biodegradation [78].

4. Experimental models to assess the bioavailability of PAHs for biodegradation

The desorption of PAHs from contaminated soils and sediments is well represented by a biphasic pattern, where there is an initial phase of rapid desorption with a rate constant that is greater than 0.1 h^{-1}, followed by a much slower phase that has desorption half-lives that may be as long as several months or even years. A slow desorption rate may be prominent if soils are enriched with semisolid components, such as NAPL-like creosote materials, which limit PAH biodegradation rates because they possess restricted surface areas and only allow the PAHs to diffuse slowly within the organic phase, and black carbon-type materials, which possess a strong sorption affinity for the PAH compounds. The desorption pattern of the PAHs can be determined by a solid-phase extraction using Tenax beads. Tenax desorption permanently reduces the aqueous concentration of PAHs to approximately zero, such that the sorption of the PAHs back to the sediment or soil can be neglected. This method is widely accepted as

a valid tool with which to assess the bioavailability of slowly desorbing PAHs, and some applications of this method have been demonstrated by our group [79, 80]. Solid-phase extraction can also be useful for determining the changes in C_{free} caused by dissolved organic matter in biodegradation studies [35]. In this approach, which is known as solid-phase microextraction (SPME), the solid extractant is applied as a silicon fiber coated by an adsorbent polymer, such as polydimethylsiloxane (PDMS), and C_{free} is determined from the HOC concentration adsorbed to the fiber at equilibrium after the system has been calibrated. These partitioning approaches can be turned into dosing methods with the use of flasks containing a film of PDMS loaded with the chemical to be analyzed [81].

The bioavailability of native PAHs present in soils and sediments can also be assessed with an accelerated biodegradation assay, characterized by a) an inoculation of a sample containing PAHs with a sufficient number of PAH-degrading microorganisms, b) a monitoring of the biodegradation activity through ^{14}C-mineralization measurements and c) a single-step chemical analysis of the native compounds in the residue. The use of ^{14}C-labelling allows for the determination of the time period required to biodegrade the bioaccessible fraction of the native chemicals. Radiorespirometry can also be easily integrated into bioavailability assessments employing chemical methods, such as solid-phase extraction, which was described in the previous paragraph. Despite the inherent difficulties in estimating the bioaccessibility of the PAHs related to the specificity of the time period and/or target organisms considered [67, 69, 82], this approach has been successfully applied to determine the recalcitrant nature of background PAH compounds found in polluted soils [83, 84], and it has also been used to determine the efficiency of bioremediation approaches designed to increase the bioavailability of aged PAHs by surfactants [80], electroremediation [85] and rhizoremediation [86].

PAHs can also be found in complex NAPLs, such as creosote and coal tar. The biodegradation of PAHs in NAPLs may be severely limited by their slow kinetics of partitioning into the water phase. The partitioning kinetics can be determined in a biphasic NAPL/water system that maintains the integrity of the organic phase, resulting in a constant interfacial area [87]. This method allows for the accurate estimations of partitioning rates and equilibrium concentrations, avoiding potential interferences resulting from the dispersion of the NAPL caused by shaking, microorganisms and biostimulants [13, 88, 89]. It also allows for a direct comparison of biodegradation rates measured under the same conditions, thus providing accurate measurements of bioaccessibility.

5. Prospective new strategies to increase the bioavailability of PAHs

An understanding of the mechanisms involved in remediation is required for the development of novel remediation technologies. While traditional approaches remove mainly the PAH fraction that undergoes rapid desorption [90, 91], the new technology should also be effective in removing the fraction that desorbs more slowly to reduce residual contaminant concentrations after treatment. Optimization efforts to remove PAHs would consider the bound fractions at a lower priority level, given the inherent difficulties associated with their measurement and because they pose low or negligible risks. The technological innovations would not only have to consider various ways of promoting phase exchanges, but they would also have to consider the risks associated with the promoting agents and/or the mobilized pollutants. Therefore, risk-minimizing strategies, such as the modulation of pollutant mobility relative to the actual biodegradation potential; the use of environmentally acceptable agents, such as surfactants, microorganisms and plants; and the application of treatment methods

requiring minimal handling to reduce the transport of the contaminants within and from the soil or sediment, should be considered.

5.1. Surfactant-enhanced biodegradation of slowly desorbing PAHs

An extensive literature review on desorption, solubilization, and the biodegradation performance of various surfactants [92-97] suggests that nonionic surfactants can increase the biodegradation rates of PAHs that desorb slowly from soils. In general, nonionic surfactants are the most frequently used types of surfactants in biodegradation studies, mainly because they are electrically neutral, which minimizes any eventual toxic effects. Compared with cationic or anionic surfactants, nonionic surfactants also generally possess lower critical micelle concentrations (CMCs). Despite their potential in bioremediation, the capacity of nonionic surfactants to enhance the biodegradation rates of the slowly desorbing PAHs remains relatively unexplored. While it is well known that nonionic surfactants promote the solubilization of PAHs present in soil [96, 97], there is a scarcity of studies that have measured the precise biodegradation rates of the slowly desorbing PAHs in the presence of surfactants. However, recent studies have shown that the use of biodegradable surfactants to improve the bioremediation performance of PAHs may be more effective in contaminated soils that have previously undergone extensive bioremediation to enrich the PAHs that desorb slowly. In a recent study, we applied a nonionic surfactant, Brij 35, to a soil originating from a site polluted by creosote and a soil from a manufactured gas plant (MGP) that had been treated by bioremediation [80]. The two soils differed in their total 3-, 4- and 5-ring PAH content (2,923 and 183 mg kg^{-1} in the creosote-polluted and bioremediated soils, respectively), but their slowly desorbing PAH content was similar (140 and 156 mg kg^{-1}, respectively). The PAHs present in the bioremediated soil

were highly recalcitrant, in spite of the presence of an active microbial population able to mineralize readily ^{14}C-PAHs (Figure 2 and Table 2). The evolution of pyrene and benzo(a)pyrene in biologically stimulated suspensions of the creosote-polluted and MGP soils showed, however, that the presence of the surfactant can influence the biodegradation of HMW PAHs in different ways. In the creosote-polluted soil, biodegradation was inhibited by the surfactant, but biodegradation in the MGP soil was enhanced by the presence of the surfactant. The different outcomes were likely a consequence of the balance of two effects, namely an increase in the bioaccessibility of the chemicals and an enhancement of the consumption rate of other PAHs present in the soil at the same time, yielding subsequent competition effects. The latter effect is in agreement with findings showing that the rate of benzo(a)pyrene co-metabolism is negatively influenced by the presence of other growth-supporting PAHs, such anthracene and pyrene, which compete for a common enzymatic pathway [98], and competition effects are also observed when growth-supporting PAHs undergo simultaneous biodegradation [14]. Another assessment was recently performed with a nonionic surfactant, Brij 30, for the treatment of contaminated soil from an MGP site that had already been treated in an aerobic bioreactor [99]. Using a single-step, slurry incubation for 18 days, the study showed that the surfactant enhanced the desorption and biodegradation of residual PAHs in the bioremediated soil, while it had no effect on the biodegradation of the PAHs in the untreated soil.

5.2. Exploiting microbial influences on PAH bioavailability

The production of surface-active compounds by microorganisms is an important microbial process that affects the bioavailability of hydrophobic organic chemicals such

as PAHs. Many different surface-active compounds synthesized by a wide variety of microorganisms, such as *Pseudomonas*, *Bacillus*, *Acinetobacter* and *Mycobacterium*, have been identified [100-102]. Despite considerable amounts of effort expended in researching these compounds, the exact physiological role that microbial surfactants play has not yet been completely elucidated, but it does not seem to be restricted exclusively to the solubilization of hydrophobic carbon sources, as surfactants can also be produced when the microorganisms are grown with water-soluble substrates, such as glucose. These biosurfactants are important in a number of ecological processes and have been linked to microbial adhesion, antagonistic effects toward other microorganisms, heavy metal sequestration and cell-cell communication. Laboratory and field studies have also shown that microbial surfactants can be successfully used for environmental applications, such as the remediation of pollution caused by heavy metals and hydrocarbons [103-106]. It is conceivable, therefore, that biosurfactants are able to improve PAH-bioremediation performance. Indeed, biosurfactants have been shown to dissolve pure, solid PAHs, such as phenanthrene, thus increasing their rate of biodegradation [88, 107, 108]. Although the production of biosurfactants is not universal among all microbes, their effect on the bioavailability of PAHs in the natural environment causes these agents to be an important factor that should be considered when optimizing the bioremediation of PAHs. In addition to biosurfactants, there is a wide variety of other natural organic compounds derived from either microbes or plants that can potentially increase the bioavailability of PAHs. For example, cyclodextrins [109] and unsaturated fatty acids [110] have been proposed to stimulate the biodegradation of PAHs in soil through this mechanism.

The bioavailability of PAHs can be increased not only by solubilizing the pollutants, but also by promoting the dispersal of microorganisms throughout the polluted matrix.

However, the positive effects resulting from the mobilization of these microbes in bioremediation operations are dependent on the efficiency of bacterial movement in porous media, which is often restricted by their high deposition rates and adhesion to soil surfaces. Bacterial active motility and taxis may help to overcome the limitation. In this review, we consider chemotaxis as the diverse tactic reactions to pollutants of bacteria exhibiting flagellar motility. Biodegradation studies based on carbon tetrachloride [111], BTEX compounds [112], pesticides [113] and one of the most water soluble PAHs, naphthalene [114, 115], have demonstrated the ability of chemotaxis to enhance biodegradation in laboratory-scale microcosms. There have been chemotactic responses demonstrated toward moderately hydrophobic PAHs, such as phenanthrene, anthracene and pyrene [116]. Chemotactic influences may be particularly important for the degradation of the most hydrophobic PAHs, such as benzo(a)pyrene, which are strongly sorbed to the soil and experience reduced mobility. However, the study on the use of chemotaxis to enhance the biodegradation of these PAHs requires new experimental and analytical methods, as well as a consideration of new concepts that have yet to be developed.

The best studied example of chemotaxis-enhanced bioavailability (and biodegradation) of a PAH relates to the capacity of *Pseudomonas putida* G7 to degrade naphthalene, and several studies have focused on this strain as a model microorganism. Using a heterogeneous aqueous system under a slow-diffusion regime, the rate of biodegradation of naphthalene by *P. putida* G7 was found to exceed the predictions from a model based on diffusion-limited biodegradation [115]. This indicated that bacterial movement through chemotaxis was faster than substrate mass transfer within the aqueous phase, thus enhancing the rate of substrate acquisition. A subsequent study that also used chemotactic and non-chemotactic strains of *P. putida* G7 clearly

demonstrated that chemotaxis increased naphthalene degradation when the compound is present in a non-aqueous-phase liquid (NAPL) [117]. In this case, chemotaxis promoted partitioning and biodegradation of naphthalene by creating a steeper gradient as the cells accumulated near the NAPL/water interface. Bioavailability can also be promoted by the chemotactic transport of *P. putida* G7 through fungal mycelia that act as pathways for mobilization [118]. This study evidenced that bacterial chemotaxis requires a liquid phase for motility. Interestingly, the liquid films surrounding fungal mycelia provide appropriate environments for chemical gradients and chemotactic responses by motile bacteria. Another study showed that the exposure regime of the chemical also appears to influence the type of tactic response [119]. Naphthalene caused in the aqueous phase chemoattraction by *P. putida* G7, but, when the chemical was exposed from a vapor phase, it acted as a repellent, even at lower doses than in the aqueous phase.

Bacterial chemotaxis has the potential to not only increase the degradation of chemoattractants, but it also has an important role in the establishment of biofilms [120] and mixed microbial communities [121] that may facilitate bioremediation. The co-adhesion and synergistic interaction of various microbial species might be strategic to improving the biodegradation of recalcitrant compounds. A technological innovation based on this concept rests on the mobilization potential of chemotactic pollutant-degrading microbes taken from plant samples [116]. We have demonstrated chemotaxis to PAHs in bacteria isolated from soil rhizospheres that were contaminated with hydrocarbons, and that these microbes are able to move chemotactically at speeds of approximately 1 mm/min [116]. It has also been shown in later research that bacterial motility and transport can be controlled through a suitable choice of chemical effectors [49, 122, 123]. In well-controlled column systems, we assessed the influence of different effectors on the deposition of a chemotactic, naphthalene-degrading bacterial

species, *Pseudomonas putida* G7, in selected porous environments (sand, forest soil, and clay aggregates). Cellular deposition, however, was concomitantly dependent on the cellular motility (hyper-motility, attraction or repulsion), the sorption of the effector to the column packing material, and the resulting pore-water concentration. For example, an exposure of the cells to salicylate induced a smooth movement with few acceleration events and positive taxis, while cells exposed to silver nanoparticles (AgNPs) exhibited tortuous movement and repulsion (Fig. 3). Although glucose was metabolized by strain G7, it did not cause any attraction, but it induced the cells to go into a hyper-motile mode, characterized by a high frequency of acceleration events, a high swimming speed (> 60 μm s^{-1}) and a high tortuosity in the trajectories. The chemically-induced motility behaviors demonstrated a distinct affinity for sand particles in batch assays, resulting in the development of breakthrough curves in percolation column experiments (Fig. 3). Salicylate significantly reduced the deposition of G7 cells in the column experiments, while glucose and AgNPs enhanced the attachment and caused a blocking of the filter, which resulted in a progressive decrease in deposition.

Microorganisms can also increase the bioavailability of PAHs when in direct contact with the pollutant, thereby enabling biodegradation to proceed more rapidly [87, 88]. The main goal of a recent study [89] was to target the potential nutritional limitations of microorganisms to enhance the biodegradation of PAHs at the interface between the NAPL and the water phase. The study found that the biodegradation of PAHs present in fuel-containing NAPLs was slow and followed zero-order kinetics, indicating bioavailability restrictions (Fig. 4). The addition of an oleophilic biostimulant enhanced the biodegradation, producing an S-shaped logistic kinetics curve, and yielded a ten-fold increase in the mineralization rates of PAHs. A chemical analysis of residual fuel oil also showed that there was an enhanced biodegradation of the alkyl-PAHs and *n*-

alkanes. This enhancement in biodegradation was not the result of an increase in the rate of the partitioning of PAHs into the aqueous phase, nor was it generated to compensate for any nutritional deficiency in the medium. These results indicate that the biodegradation of PAHs by bacterial cells attached to NAPLs can be limited by nutrient availability as a result of the simultaneous consumption of PAHs within the NAPLs, but this limitation can be overcome by interface fertilization.

5.3. Rhizoremediation

Rhizoremediation is the use of ecosystem services provided by plant rhizospheres to decontaminate polluted soils, and it has recently gained attention with regard to the remediation of organic pollutants such as PAHs. The high levels of microbial biomass associated with the rhizosphere, the carbon turnover caused by root exudates and their microbial utilization as co-substrates for the co-metabolism of PAHs and chemotaxis, as well as the migration of dissolved contaminants within the soil matrix as a result of interactions with DOM, are all positive factors that promote the bioavailability of these PAHs. Plants utilized for the rhizoremediation of PAHs must have developed an extensive root system and a strong positive geotropism to achieve the maximum colonization in the contaminated area, and it is important for the plant to be able to tolerate the presence of these contaminants. Some plant species appear to be more successful in stimulating the biodegradation of PAHs than others; therefore, it is critical to select an optimal plant for rhizoremediation. A significant number of studies have been conducted on grasses from the Graminaeae family, mainly tall fescue (*Festuca arundinacea*), switchgrass (*Panicum virgatum*) and ryegrass (*Lolium perenne*) [65, 124-127]. The sunflower (*Helianthus annuus*), an annual plant belonging to the Asteraceae

family, has also been used by several authors as a model plant. The sunflower rhizosphere removed a greater quantity of fluorene, anthracene and pyrene from contaminated soil than the rhizospheres belonging to other plant species, such as wheat, oat and maize, and responded better in terms of seed germination and root elongation in the presence of these PAHs [128]. In another comparative study, the sunflower was also reported to be the best plant among 11 dicotyledonous species in promoting the dissipation of aged PAHs from soil [125]. Other advantages to focusing on the sunflower as a model plant for the rhizoremediation studies of PAHs include the importance of this species as a producer of biofuel. The ability to investigate the root exudation process and the role of the compounds that are exuded under natural conditions has been hampered by a number of significant quantification problems, such as interference by microbial metabolites and other components found within the soil. These problems can be overcome through the development of appropriate *in vitro* techniques to obtain root exudates that allow for an analysis of the products secreted by the plant roots. As the sunflower is an important edible oil producer, its response to propagation and in vitro tissue culture is also widely known [129].

In a recent study [86], we employed a soil that was polluted with aged PAHs at concentrations considered realistic for soils that have undergone extensive bioremediation to test the hypothesis that the germination and development of sunflower plants would enhance the bioavailability of PAHs in the soil. We determined the effect of planting sunflowers on the dissipation of the PAHs from the soil under greenhouse conditions. We also developed a method to produce sunflower root exudates, which were chemically characterized and tested for their effects on the biodegradation of PAHs. We observed that the dissipation of PAHs from soil promoted by sunflower plants after the greenhouse experiment had been run for 90 d could be

reproduced over a shorter period of 10 d by incubating the soil in shaked slurries and adding root exudates (Table 3). The concentration of total organic carbon (TOC) from the root exudates found in this study was 129.73 mg L^{-1}, comparable to TOC values reported in other studies on the promoting effects of root extracts on PAH-degrading microorganisms. For example, Rentz et al. [130] reported TOC concentrations of 84.2, 175.0 and 51.7 mgL^{-1} from the root extracts of hybrid willow (*Salix alba x matsudana*), kou (*Cordia subcordata*) and milo (*Thespesia populnea*), respectively, while Miya and Firestone [131] reported a TOC concentration of 54 mg L^{-1} for slender oat root exudates. It is possible that the organic carbon in the exudates enhanced the bioavailability of PAHs by a mechanism related to its ability to mobilize PAHs that were initially sorbed to the soil, as it is known that the addition of dissolved organic matter (DOM) to contaminated soils causes an enhanced biodegradation of PAHs, which is probably a result of an enhanced desorption of the PAHs. The chemical characterization of the root exudates also identified specific substances with the potential to directly increase the bioavailability of the PAHs. These substances include chemicals that are able to induce chemotaxis. For example, sugars, such as fructose, have a positive chemotactic effect on soil microorganisms [132]. Amino acids, such as glutamine, aspartic acid and isoleucine, which were also found to be components of sunflower root exudates in this study, are powerful chemoattractants for *Rhizobium* and *Bradyrhizobium japonicum* [133]. Zheng and Sinclair [134] indicated that alanine, asparagine, glutamine, serine, and threonine in soybean root exudates may serve as chemoattractants for the *Bacillus megaterium* strain B153-2-2. Finally, we detected fatty acids, such as palmitic acid and stearic acid, which are plant components with a known potential to enhance the bioavailability of PAHs in soil by acting as surfactants [110]. Vegetable oils have also been widely used as natural organic solvents [135, 136],

resulting in the dissolution of PAHs and consequently enhancing the biodegradation of these PAHs. Therefore, the presence of these compounds may explain the greater decrease in PAHs observed when sunflowers are used for soil treatments.

5.4. Electrobioremediation

The use of electroremediation for the treatment of PAH pollution has recently been addressed as a relevant innovative step in soil remediation [137]. However, to the best of our knowledge, there have been limited attempts to utilize this technology for the bioremediation of PAHs. The application of an electric current to the soil may lead to a number of simultaneous processes and changes occurring within the soil, which may promote the biodegradation of organic pollutants, such as the migration of ions (e.g., Ca^{2+} and PO_4^{3+}), and changes in the soil moisture content and/or dissolved oxygen content. Our results indicate that electrokinetics can also be successfully employed to enhance the bioavailability of PAHs, particularly for the bioremediation of soils that are rich in clay fractions and/or aged contaminants [52, 85, 138, 139].

The electrokinetic mobilization of PAHs in soils has been observed in several studies that used phenanthrene as a model PAH and solubility-enhancing agents, such as surfactants, co-solvents, and cyclodextrins, in the flushing solutions. These studies have shown that, despite the limited solubility of phenanthrene, which restricts its transport through the soil, it can be mobilized even in the absence of these agents. For example, Saichek and Reddy [140] observed that there was a nearly complete removal of phenanthrene from kaolin soil in the region adjacent to the anode when the soil was flushed with deionized water after undergoing electrokinetic treatment. However, the phenanthrene concentration profile in the rest of the soil remained unaltered. A significant removal of phenanthrene (by up to 35%) was reported in another study

where kaolinite samples were initially amended with phenanthrene and treated electrokinetically with a purging solution containing NaCl [141]. In an electrobioremediation study [52], we observed that the rate of phenanthrene removal during treatment in the absence of any solubilizing agent was ten-fold higher in soil that had been treated with an electric field than in the control cells that were not supplied with any electric current or microbial activity, where there was a negligible removal of phenanthrene. Despite the relatively high concentration of phenanthrene in the soil (approximately 1,300 mg kg^{-1}), the biodegradation kinetics was observed to be first-order in separate biodegradation experiments, which is indicative of a concentration-dependent biodegradation [12]. It is therefore possible that the electrokinetic treatment caused a mobilization of phenanthrene toward bacterial cells, thus contributing to an increased localized concentration of phenanthrene, leading to a higher biodegradation rate of the compound within the soil. With a typical microbial population density of 10^7 to 10^8 cells g^{-1}, the colonies of degrading microorganisms in soil are separated by an average distance of 100 μm; thus, they are surrounded by a desolate environment where the biodegradation rate is controlled by the dissolution and diffusion of these hydrophobic pollutants from distant sources [142]. According to this mechanism, a significant electro-osmotic mobilization of phenanthrene at the macroscale level would not have been needed for this observed stimulation to occur.

An electrokinetic pre-treatment has also been evaluated as a way to mobilize the less bioaccessible fraction of PAHs [85, 139]. These studies incorporated a surfactant, Brij 35, to optimize the process performance. The residual concentrations of the total biodegradable PAHs remaining after bioremediation in soil slurries were two-fold lower in electrokinetically pre-treated soils than in untreated soils (Table 4). It is conceivable that physicochemical changes produced in polluted soil particles that were exposed to

electric fields may promote the bioavailability of these PAHs, thus improving the bioremediation performance. The study suggests that the application of an electric field to the soil in the presence of the nonionic surfactant Brij 35, through the electroosmotic flow that was consequently generated, modified the bioavailability of the PAHs by changing their desorption kinetics. The development of an electroosmotic flow through soil aggregates could probably have caused an increase in the rate of slow desorption, thus increasing bioavailability [143]. The induced physical and chemical changes could have resulted in the mobility of the pollutant fraction entrapped within the soil nanopores and/or strongly sorbed to black carbon.

6. Implementing bioavailability-promoting innovations in bioremediation

In this review, we have seen a wide array of innovations targeted at increasing bioavailability while not necessarily increasing the environmental risk. From the experience already gained in the science and technology of PAH bioremediation, the integration of these new achievements seem promising. For example, the selective use of surfactants in soils enriched in PAHs that desorb slowly would not only avoid the inhibition of HMW PAH biodegradation through competition mechanisms. This strategy would also allow for the minimization of risks associated with an increased chemical activity and toxicity of the HMW PAHs and metabolites as a result of solubilization, at concentrations in excess of the metabolic potential of the microorganisms. Other advantages of this different way of surfactant application may relate to the biodegradable nature of the surfactant, which is obviously necessary to minimize environmental impacts. The use of a surfactant involves eventual increases in nutrient and oxygen demands, which would be less important when pollutant loads are attenuated and less bioaccessible, as well as the loss of surfactant after extended

biodegradation periods, when it is required to solubilize the slowly desorbing PAHs. However, the use of surfactants in environmental remediation may be potentially risky because they may be toxic and harm the soil quality, leading to the need for other economic considerations. As a result, it may potentially be better to use biosurfactants or other naturally occurring stimulants. However, the effects of these biostimulants on the biodegradation of slowly desorbing PAHs or NAPL-associated PAHs remain largely unknown. Moreover, the cost of producing natural biostimulants at a large scale for environmental applications remains an unanswered question.

The new knowledge of bacterial chemotaxis and adhesion may have implications for the design of new bioaugmentation and biostimulation strategies oriented toward improving the performance of PAH-degrading microbial populations. These strategies may overcome, for example, the limitations usually caused by the addition of inoculants designed only on the basis of their catabolic potential. A treatment of extended areas of contamination and pollutants present at a certain depth can benefit from the use of positive chemical effectors that increase the outreach of these introduced inoculants via chemotaxis. For localized and surface contaminations of soils and aquifers, negative chemoeffectors and alternative carbon sources that cause hyper-motility would be useful in rapidly confining motile bacteria within the contaminated site, and they would be especially useful for the treatment of aged PAHs by promoting the association of bacterial cells with the solid phase of the contaminated materials. The protective effect of fungal propagules, such as the mycelia, oospores and zoospores on bacterial cells, may also aid in dispersing mixed fungal/bacterial inoculants and allowing for the colonization of specific niches in soil that are not suitable for bacterial growth on their own. Interface fertilization strategies may be relevant for bioremediation scenarios that require the treatment of subsurface NAPL sources of pollutants, which is often limited

by the slow kinetics of PAH partitioning into the water phase, the difficulties in localizing the NAPL sources, and the risks of contamination to underground water caused by the mobilization of the pollutants.

Rhizoremediation and electroremediation provide ways to increase the bioavailability of PAHs in a low-risk manner through different mechanisms. Plant rhizospheres could be effective in promoting the bioavailability of PAHs in contaminated soils that have previously undergone extensive bioremediation but that still contain unacceptable PAH levels. When taking into consideration the agronomic advantages of certain plant species, such as sunflowers, in relation to the knowledge acquired for their exploitation and their potential as biofuel producers, rhizoremediation seems to represent a promising alternative for increasing the bioavailability of PAH compounds in a sustainable and low-risk manner. The benefits derived from utilizing the potential of the relatively new electrobioremediation technology will depend on the type of soil; for example, clay-rich soils exhibiting a high consistency, nanoporosity and surface area available for sorption may be more difficult to treat biologically than sandy soils, but their treatment can be made more affordable by this combined approach. The successful bioremediation of PAH-polluted soils rich in black carbon, such as manufactured gas plant soils, may also be limited by bioavailability restrictions that can be overcome through the use of this hybrid technology. The treatment of highly heterogeneous soils, where different materials may possess highly dissimilar hydraulic conductivities, may also benefit from electroosmotic flow, which is not affected by the pore size distribution of the soil and the presence of macropores in the soil.

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Figure Legends

Fig. 1. Conceptual framework of this review. Bioavailability, bioremediation and environmental risk of polycyclic aromatic hydrocarbons (PAHs, represented by the benzo(a)pyrene molecule in the centre) are interconnected through a variety of concepts related to technological performance, environmental microbiology and organic chemistry, and risk assessment.

Fig. 2. Effect of an anionic surfactant (Brij 35) on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in soil slurries. Mineralization of ^{14}C-phenanthrene (left) and ^{14}C-pyrene (right) in the absence (squares) and presence (circles) of Brij 35. Top, creosote-polluted soil slurries from Andujar, Southern Spain. Bottom, slurries with soil from a manufactured gas plant site in Denmark, previously bioremediated (Soilrem). These experiments were run in parallel to the determination of the native PAHs (Table 2). Modified with permission from [80].

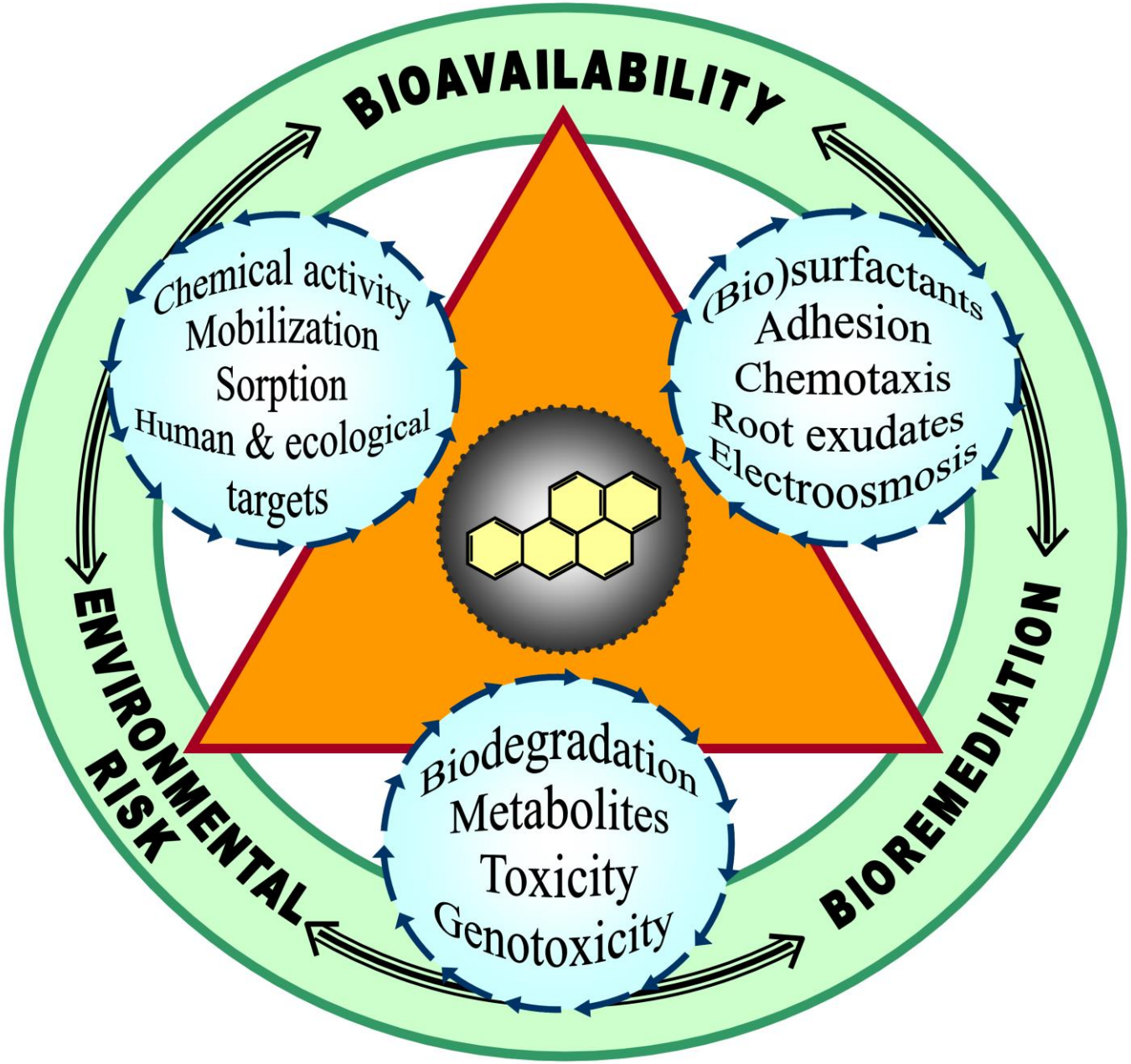
(Note to technical Editor: Figure 2 is meant to be composed in the same page together with Table 2)

Fig.3. Effect of exposure of naphthalene-degrading *Pseudomonas putida* G7 to salicylate (promoting positive taxis) and to silver nanoparticles (repellence) on bacterial transport through sand (a) and swimming behavior (b-d). Salicylate significantly reduced deposition of G7 cells, whereas AgNPs enhanced attachment and caused filter blocking that resulted in a progressive decrease in deposition (a). In experiments designed to test the effects of these chemicals on single cell motility, computer-aided analysis of cell trajectories showed (c-d) that exposure to salicylate

induced smooth cell movement with no turning events (peaks above 1000 deg s^{-1} in the rate of change of direction or RCDI, indicated by asterisks), what is characteristic of positive taxis. Cells exposed to AgNPs exhibited tortuous movement, built on a high frequency of turning events; this behavior is attributable to cells sensing a repellent. Both tactic responses were confirmed by separate capillary assays. Modified with permission from [122].

Fig.4. Effect of a oleophilic biostimulant (S-200) on mineralization of phenanthrene in a nonaqueous-phase liquid (NAPL). Symbols represent percent ^{14}C mineralized without biostimulant (white circles) and with biostimulant (drak squares). The dashed line represent biodegradation predicted by maximum abiotic partitioning rate determined in the absence of bacteria. The NAPL was a mixture of heavy fuel oil and heptamethylnonane. Modified with permission from [89].

1 **Fig. 1.**


BIOAVAILABILITY
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Sorption
Human & ecological
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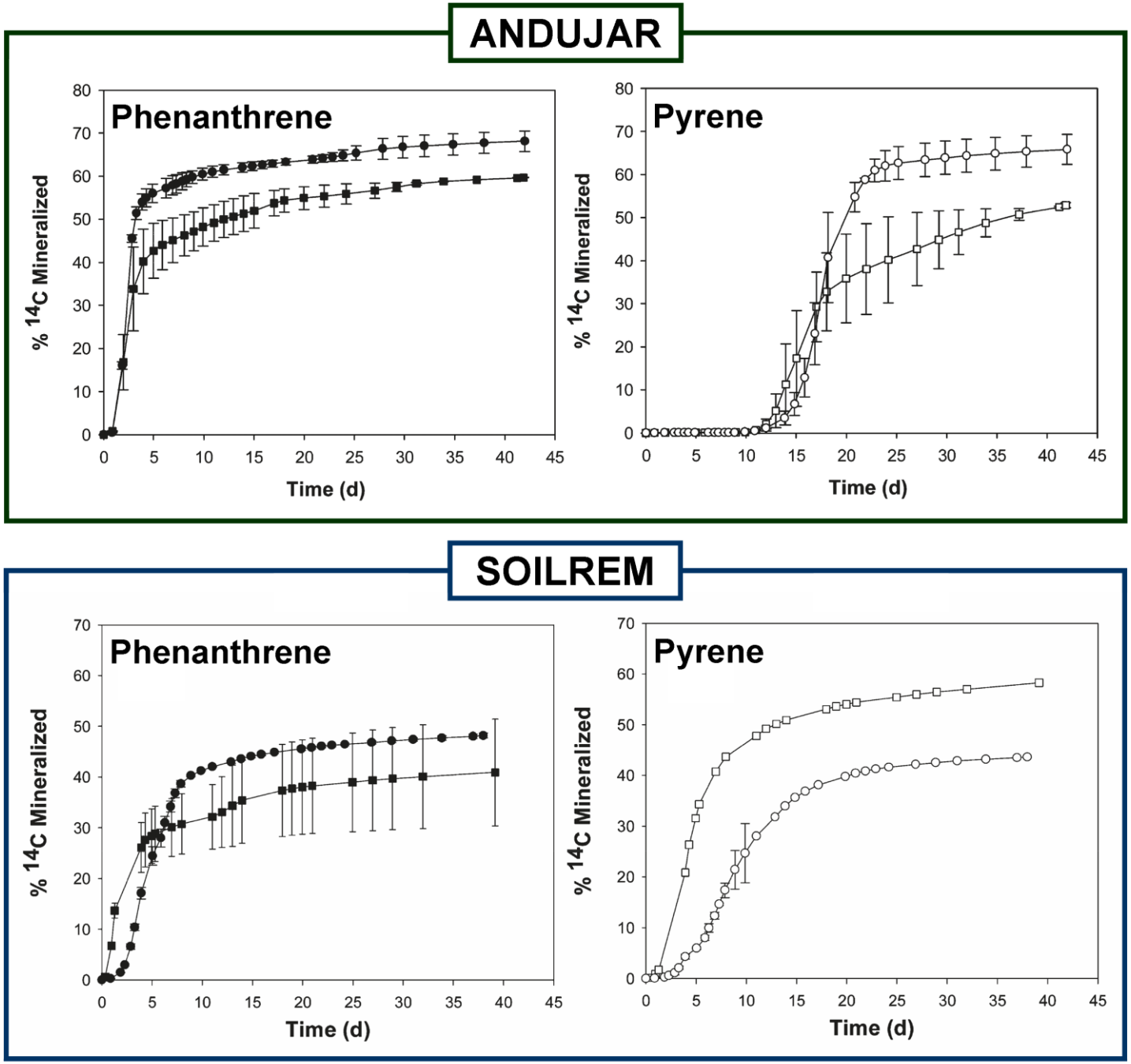
Adhesion
Chemotaxis
Root exudates
Electroosmosis
Biodegradation
Metabolites
Toxicity
Genotoxicity
ENVIRONMENTAL
RISK
BIOREMEDIATION

1 **Fig. 2.**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Pyrene
% 14C Mineralized
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Fig.3

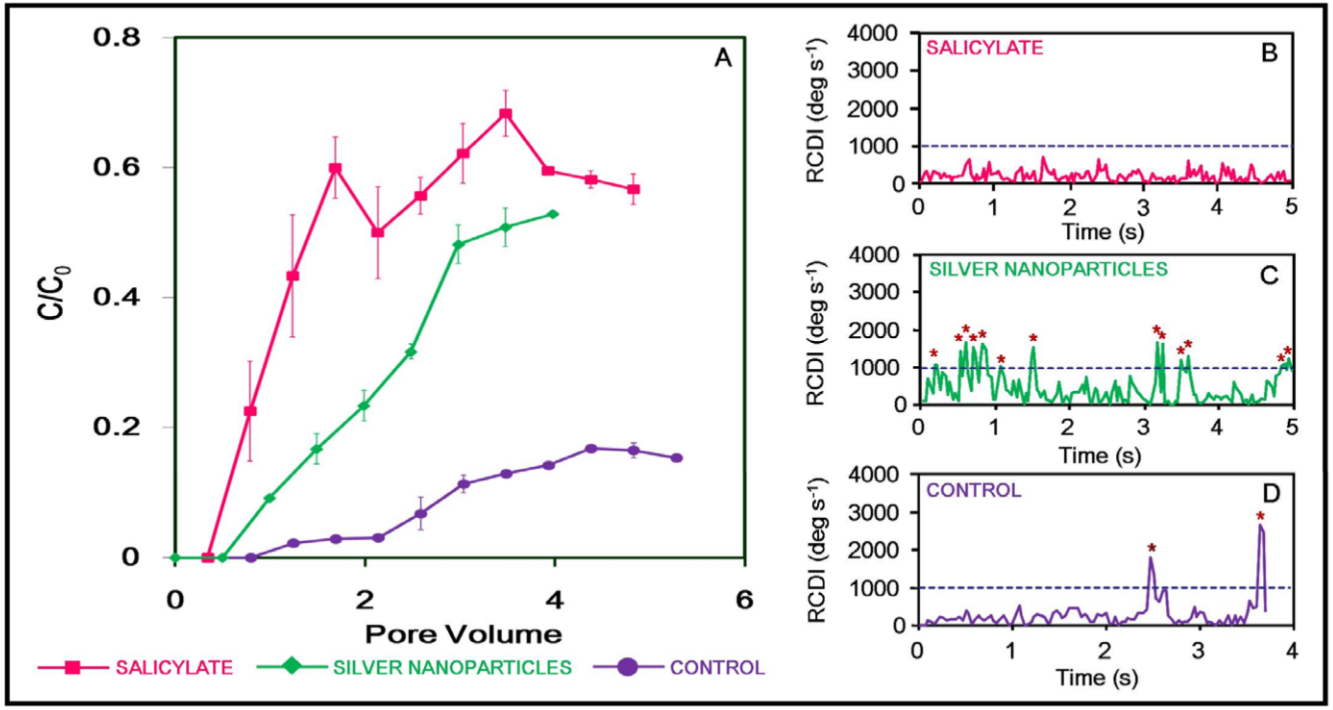
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Fig. 4.

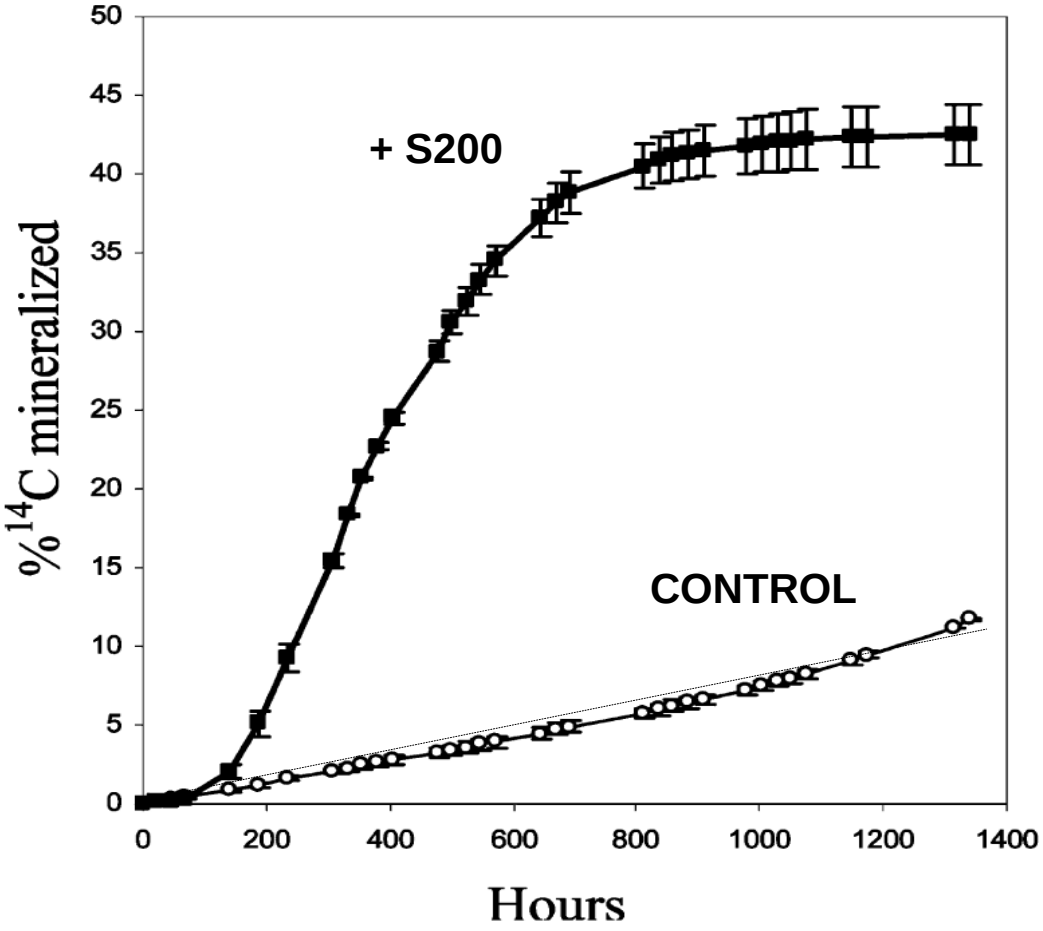
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Table 1. Bioremediation technologies, available at commercial stage, for treatment of pollution by polycyclic aromatic hydrocarbons.

Technology	Description	Example
Land farming	*In situ* periodical tilling and fertilization with N and P	[61]
Composting	Amendment with biodegradable organic materials, fertilization and pile maintenance under controlled humidity and aeration	[62]
Prepared-bed bioreactor	Pile treatment with recirculation of irrigated water and nutrients	[63]
Slurry-phase bioreactor	Ex situ mixing with liquid phase, controlled aeration	[64]

3

1
2
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Table 2. Effect of an anionic surfactant (Brij 35) on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in soil slurries.[a]

	PAH	Native PAH concentration (mg kg^{-1})			
		Initial	Predicted[b]	Control[c]	+Brij35[c]
ANDUJAR	Phenanthrene	1329	52	9	<LD[d]
	Pyrene	395	30	18	25
	Benzo(a)pyrene	31	4	17	28
	∑PAH[e]	2923	140	111	76
SOILREM	Phenanthrene	42	37	43	18
	Pyrene	48	41	38	8
	Benzo(a)pyrene	22	19	22	10
	∑PAH[e]	183	156	167	69

[a]Data of native PAHs obtained from experiments run in parallel to radiorespirometry assays shown in Fig. 2.
[b]Predicted concentration assuming that biodegradation acted only upon fast-desorption PAHs. Calculated with the values of fast-desorbing fraction or F_{fast} as determined with Tenax extraction.[c]Mass balance of the native compound remaining in the soil and in the aqueous phase at mineralization plateaus in Fig. 2.
[d]<LD, below limit of detection.
[e]Sum of five PAHs: phenanthrene, anthracene, fluoranthene, pyrene and benzo[a]pyrene.

Source: Modified with permission from [80]

Table 3.Effect of planting with sunflowers and slurry-phase treatment with exudates on residual contents of polycyclic aromatic hydrocarbons (PAHs) in polluted soil.[a,b]

PAH	Greenhouse			Slurry		
	Initial	Control	Planted	Initial	Control	Exudates
Phenanthrene	4.0	0.5	0.1	4.6	1.6	1.0
Pyrene	2.0	0.3	0.1	1.0	0.5	0.2
Chrysene	1.3	1.0	0.5	1.5	0.8	0.5
∑PAH[c]	21.8	5.0	1.5	19.3	4.8	2.5

[a]Data of native PAHs in mg kg^{-1} obtained from greenhouse and bioaccessibility experiments. As biodegradation was tested successively, initial and control data are reported for each experiment separately.
[b]Greenhouse and slurry indicate, respectively, whether the polluted soil was planted with sunflower and maintained during 90 d under greenhouse conditions, or shaken in a slurry under laboratory conditions during 10 d in the presence of sunflower exudates produced previously *in vitro*.
[c]Sum of six PAHs: fluorene, phenanthrene, anthracene, fluoranthene, pyrene and chrysene.

Source: Modified with permission from [86]

Table 4. Effect of electrokinetic treatment on the bioaccessibility of polycyclic aromatic hydrocarbons (PAHs) in slurries of creosote-polluted clay soil and loamy sand, as compared with an untreated control.[a,b]

	PAH	Control		Electrokinetic treatment		
		Initial	C_f	Initial	C_e	C_f
CLAY SOIL	Phenanthrene	159	14	162	110	4
	Pyrene	47	9	64	52	4
	Benzo(a)pyrene	2	2	5	6	2
	∑PAH[c]	287	63	363	290	29
LOAMY SAND	Phenanthrene	106	3	164	73	2
	Pyrene	30	4	54	26	1
	Benzo(a)pyrene	2	1	4	2	1
	∑PAH[c]	210	18	369	173	11

[a]Data of native PAHs in mg kg^{-1} obtained from bioaccessibility experiments run in parallel with radiorespirometry assays. As bioaccessibility was tested successively, control data are reported for each experiment separately.
[b]C_f (PAH concentration after bioaccessibility assay), C_e (PAH concentration after 7 days of electrokinetic treatment).
[c]Sum of five PAHs: phenanthrene, anthracene, fluoranthene, pyrene and benzo[a]pyrene.

Source: Modified with permission from [85]